**Translational Approaches for Cystinosis Targeting Autophagy**

**Sergio D. Catz, Ph.D.**
Department of Molecular and Cellular Biology
The Scripps Research Institute, La Jolla, California

Lysosomes regulate cellular homeostasis and autophagy. Cystinosis, a lysosomal storage disease caused by defects in the cystinosin gene (CTNS), results in cystine accumulation, primarily affecting the kidneys and ultimately leading to chronic kidney disease (CKD). We previously demonstrated that cystinosis is characterized by impaired chaperone-mediated autophagy (CMA) resulting from decreased expression and mislocalization of the lysosomal receptor LAMP2A. This is associated with the accumulation of misfolded proteins and endoplasmic reticulum (ER) stress. Macroautophagy is also disrupted in cystinosis, contributing to cellular stress and tissue dysfunction.

We demonstrated that CA77, a RAR-α inhibitor and CMA activator, restores LAMP2A levels and enhances renal function in *Ctns*$^{-/-}$ mice. To accelerate translation, we screened the ReFRAME repurposing library (~13,000 compounds) using an in-silico approach, identifying 84 RAR-α inhibitor candidates. Further validation using a reporter cell-based screening yielded 11 hits. One of these compounds, C-5, significantly upregulated LAMP2A and the transcription factor TFE3 while decreasing LAMP1 and LC3B-II expression, thereby effectively restoring the autophagy balance and reducing lysosomal stress.

Increasing LAMP2A expression enhances CMA, contributing to the clearance of misfolded proteins and reducing ER stress. TFE3 activation promotes lysosomal biogenesis and regulates autophagy, thereby improving lysosomal function. Conversely, a decrease in LAMP1 indicates reduced lysosomal overload, while decreased LC3B-II levels suggest increased autophagic flux. Taken together, our data indicate that C-5 improves proteostasis and reduces cellular stress in cystinosis. *In-vivo* validation is underway to study the therapeutic potential of C-5 in cystinosis.